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Research Article

Role of Fluvastatin sodium loaded polymeric nanoparticles in the treatment of Hyperlipidemia: Fabrication and Characterization

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Abstract

The present work is focused on the formulation and evaluation of polymeric nanoparticles (NPs) loaded by Fluvastatin sodium (FLS). This drug has many disadvantages, including bioavailability of up to 24 to 44% and a half-life of approximately 30 min to 60 min. Also, it comes under Biopharmaceutical classification system (BCS) class III, but it is sparingly soluble in water. Polymeric NPs were prepared using gum rosin and chitosan as polymers which Solvent Evaporation and Inotropic Gelation method prepared. Prepared polymeric NPs were characterized for respective parameters like drug entrapment efficiency, particle size, zeta potential, Differential Scanning Calorimetry, X-Ray Diffraction, Scanning Electron Microscopy, Transmission Electron Microscopy, In vitro and In vivo drug release study. The average Particle size was lay within the range of 271 nm to 313.3 nm for the Solvent Evaporation method (SE) and 123.3 nm to 382 nm for the Inotropic gelation method (IG). Drug entrapment efficiency (DEE) of SE3 was found to be 86.1%, and IG3 had 70.2%. In vitro release study showed sustained action for both methods at the end of 48 h; an increase in the polymer resulted in decreased drug release. From the study, it can be concluded that prepared polymeric NPs from both methods show significant hypolipidemic activity. Stability studies showed that formulations are stable at the end of 3 months.

Keywords: Fluvastatin sodium; solvent evaporation method; inotropic gelation method; drug entrapment efficiency; polymeric nanoparticles

Introduction

Nowadays, the most vitality-possessed branch is nanotechnology: which provides an opportunistic pathway for various fields like biomedicine, medicine, pharmacy, biotechnology, engineering, chemical engineering, and polymer science. It plays a significant role in pharmaceutical sciences as a tool for diagnosis and treating aid [1]. Nano is a Latin word whose meaning is 'dwarf'. Nanotechnology involves a complete size range, one thousand millionths of a meter, i.e., 1 nm = 10^{-9} meters. Nanotechnology is a branch of science that studies processes occurring at the molecular level and non-lengthy scales. According to various literature, the term NPs refer to particulate dispersions, solid dispersions, or solid particles with a size from 10 nm to 1000 nm in which entrapped/encapsulation or attachment of drug can be performed [2].

Recent studies reveal that polymeric NPs have manifested several new properties and activities due to their nanoscale size and stability. Polymeric NPs ranging from 1 to 1000 nm can carry active compounds entrapped inside or surface adsorbed onto their core [3]. The size of polymeric NPs lies between 20 and 250 nm. They can be classified into three types based on how they have been encapsulated: Linear polymeric NPs – covalent for drug conjugation, micellar polymeric NPs –

amphiphilic block copolymers involved in the formulation, Hydrogels – encapsulation of hydrophilic drug [4].

Pharmaceutical formulations that contain NPs formulated with biopolymers are gaining importance. A particular interest is paid to polysaccharide NPs due to their multifunctional, nontoxic, and biocompatible properties. Since they usually have multiple functions, they improve the permeability and solubility of many powerful drugs. The penetration strength of polysaccharide–loaded drugs inside tissues and their biocompatibility make them more effective [5]. Chitosan is the most abundant natural polysaccharide. This is the most important naturally occurring cationic polymer approved for tissue engineering, drug delivery, and gene transfer by the FDA and EMA [4,6]. Gum rosin is a thick resin produced by pine trees and coniferous plants. Acid and modified acid forms of resins, like dimers and decarboxylated acids, are present. They provide better biocompatibility and biodegradability [5].

Since the beginning of time, humans have been affected by hyperlipidemia. A systemic correlation between blood lipids, hyperlipidemic conditions, and their complications was established in 2002 by coronary heart epidemiology studies [7]. Various inherited and acquired disorders result in hyperlipidemia, indicated by raised levels of lipids in the body. Across the globe, it is a common disorder, which is especially prevalent in the western hemisphere. Because this disorder usually does not have symptoms, it is likely to be diagnosed early and prevent the development of the disease [8].

As the first synthetic statin, FLS is used in treating hyperlipidemia as the first-line therapy. Bioavailability is up to 40 % for this amorphous powder. FLS has a relatively short half-life of 3 h and belongs to BCS Class III. The substance is wholly absorbed even when food is present. A single drug dose is excreted nearly 95% via the biliary route and less than 2% as the parent compound. The standard dose of FLS is 8-10 mg per day and the log P value is 3.86. FLS is therefore selected as the best candidate for fabricating polymeric NPs using solvent evaporation and inotropic gelation using biodegradable polymers like gum rosin and chitosan to approach sustained release action [9,10].

Material and methods

Pure FLS drug was gifted as a sample from Biocon ltd. Bangalore. Gum rosin (**CAS NO** – 8050097, 98% pure, Sigma Aldrich, Bangalore Karnataka), polyvinyl alcohol (35 – 50 cps, **CAS NO** – 9002895, 98% pure, Fischer scientific, Mumbai, Maharashtra), Ethanol 95% (**CAS NO** – 64175, 99 % pure, CSS chemical co. Ltd, Mumbai, Maharashtra), Chitosan from Shrimp shells (**CAS NO –** 9012764, 99% pure, Loba Chemie, Mumbai, Maharashtra) and Sodium tripolyphosphate (STPP) (**CAS NO** – 7758294, 99% pure, Fischer scientific, Mumbai, Maharashtra) were used in the preparation of NPs. All the ingredients used in the preparation of polymeric NPs possess analytical grades.

Table 1. Formula for the preparation of polymeric NPs by the solvent evaporation method.

		Formulation code		
Sl. no.	**Ingredients**	**SE1**	**SE2**	**SE3**
1	FLS (mg)	100	200	100
2	Gum Rosin (mg)	300	400	400
3	Ethanol (ml)	10	10	10
4	Polyvinyl alcohol (mg)	500	500	1000
5	Distilled water (ml)	100	100	100

Fabrication of polymeric NPs by the solvent evaporation method

Formulation contents are mentioned in Table 1. Accurately weighed FLS and gum rosin were dissolved in ethanol to obtain solution I. Simultaneously polyvinyl alcohol was added to distilled water to obtain solution II. This surfactant solution was homogenized for 5 min at 25000 rpm to dissolve Polyvinyl alcohol. Solution I was slowly added to solution II. Then it was subjected to homogenization for 10 min at 15000 rpm. The dispersion was sonicated for 5 min (15 cycles with 30 sec of halt). Each formulation was stirred at a magnetic stirred at 100 rpm for 6 h to evaporate ethanol, centrifuged at -20°C at 15000 rpm for 20-30 min, and the supernatant liquid was discarded to obtain pellets. The pellet was redispersed in cryoprotectant (0.5% mannitol) solution and pre-frozen at pre glycolic path at -40°C.

It was lyophilized at -80°C for 4 h (primary drying) and -40°C for 24 h (secondary drying). The obtained spongy NPs were stored in an air-tight container to avoid moisture [11].

Fabrication of polymeric NPs by inotropic gelation method

Ingredients required for the formulation are mentioned in Table 2. 0.1%, 0.2%, and 0.3% of chitosan solution (chitosan of shrimp shells) were prepared by using 1% v/v acetic acid solution and subjected to sonication for 20-30 min to obtain a clear solution (if not filter with muslin cloth). Then the pH of the chitosan solution (pH 3.4) was altered to 5 to 6 by adding 0.1N NaOH solution. STPP solution was added in deionized water/ distilled water at various concentrations of 0.1%, 0.15% and 0.20% while stirring at 750 rpm for 30 min to get respective STPP solutions. FLS was dissolved in a 1:1 ratio of Ethanol: Water to obtain a clear solution. This solution was added dropwise with a #26 syringe (0.45 mm) to 40 ml of chitosan solution and sonicated for 1 h cycles. 20 ml of STPP was added 0.75ml/min under stirring at 1000 rpm at 25°C and stirred for 30 min. Further, it was bath sonicated for 30 min at 50 HZ and centrifuged at 9000 rpm for 2 h. Pellets were dispersed in 2% mannitol, pre-frozen at -40°C for 15-30 min, then lyophilized at -40°C for 24 to 48 h [12].

Table 2. Formula for the preparation of polymeric NPs by inotropic gelation method.

Sl. no.	Ingredients	Formulation code		
		IG1	IG2	IG3
1	FLS (mg)	100	100	100
2	Ethanol: Water (ml)	1:1	1:1	1:1
3	Chitosan (mg)	100	200	300
4	Acetic acid 1% v/v	1%	1%	1%
5	Sodium tripoly phosphate (STPP) (mg)	100	150	200
6	Distilled water (ml)	100	100	100

Characterization

Solubility study of polymeric NPs

According to the drug solubility profile of FLS, the polymeric NPs prepared from both methods was subjected to a solubility test by using solvents in which FLS drug is soluble, like distilled water, methanol, ethanol, and DMF. The sample equivalent to 1 mg was dissolved in 10 ml of solvent and sonicated for 2 min [13].

Drug entrapment efficiency

Solvent evaporation method (SE)

NPs equivalent to 5 mg of FLS were taken, and 80 ml of 7.4 pH buffer was added. It was kept aside overnight by sealing it with silver foil. It was stirred and heated gently at 40°C for up to 15 min. The rest of the 20 ml buffer was added. Further, aliquots were prepared according to Beer's range, and absorbance was measured at 305 nm spectrophotometrically [11,12].

Inotropic gelation method (IG)

NPs equivalent to 5 mg of FLS was dissolved in a 7.4 pH buffer with continuous stirring. The solution was filtered through 0.45 micron-sized filter paper. It was diluted according to Beer's range, and measurement was recorded spectrophotometrically at 305 nm [10]. The calculated according to the formula:

$$\% \text{ DEE} = \frac{\text{content of drug practiclly obtained}}{\text{Theoratical drug calcuated}} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

After a baseline correction with dried potassium bromide to confirm compatibility, FTIR analysis was carried out on purified drug and NPs obtained by mixing with Potassium Bromide (KBr). In an evacuated die, pellets were pressed to 5 x 106 Pa to produce clear transparent pellets with an approximate diameter of 1.5 to 2 cm and thickness of 0.2 cm. An integrated Fourier transform

spectrometer (Bruker, USA) was used to record spectra at room temperature, scanning between 4000 cm^{-1} to 400 cm^{-1} [14].

Characterization by Particle size, Poly dispersity index, and Zeta potential

The average particle size, poly dispersity index, and zeta potential of the fabricated NPs from both methods were measured by a particle size analytical instrument (Nano Zeta Sizer, Malvern Instruments, UK) using techniques of Dynamic Light Scattering for size analysis and combination of Laser Doppler Velocimetry and Phase Analysis Light Scattering for zeta potential. Values of these both give Polydispersity index (PDI) [15].

Transmission electron microscopic studies (TEM)

Using a JEOL JEM 2010F UHR operating at 200 kV, HR-TEM photographs were obtained for polymeric NPs from SE2. The technique involves preparing the suspension of NPs in polyol obtained from the SE method, then placed on the amorphous carbon membrane of the transmission electron microscope grid and allowing it to evaporate solvent at room temperature. In order to eliminate most of the organic molecules, the grid was again thermally treated under an ultrahigh vacuum at 150 °C. With this treatment, high-resolution images were greatly improved without modifying particle crystallinity. We analysed the TEM images with a digital camera and the SAISAM and TAMI's software (Micro vision Instruments) to determine the particle size distribution [16].

Scanning electron microscopic studies (SEM)

NPs of IG3 were coated with a thin gold layer by sputtering. The microstructure was observed in a scanning microscope attached to an energy dispersive analysis of X-ray (EDAX) instrument (Joel 6390LA/ OXFORD XMX N) that operated at an acceleration voltage of 20 kV [17].

Differential scanning calorimetry (DSC)

DSC thermograms of pure medicines and NPs were taken using heating rates of 10 K/min from 20°C to 300°C in 25 ml/min under nitrogen flow. The aluminium crucibles were covered with a perforated lid before the samples were sealed. An empty aluminium crucible was used as the reference [18].

X-ray diffraction (XRD)

At room temperature, XRD patterns were collected using a Cu-Kα radiation diffraction system (Bruker D8 Advance diffractometer). Using a LYNXEYE XE-T detector and Kβ radiation which has monochromaticity, the instruments were utilized to scan samples of pure drug and NPs over 2-degree ranges of 3-50°C [19].

In vitro drug release study

In vitro drug release study of polymeric NPs was performed by diffusion study, i.e., equilibrium dialysis membrane method. The experiment was performed in a 1.2 pH buffer for 2 h and then using a buffer solution of pH 7.4 for the rest of the hours (till the end). 10 mg FLS equivalent to polymeric NPs (Equivalent to the dose of the drug) was taken and dissolved in 10 ml of methanol for the IG method and 10 ml of distilled water for the SE method. This suspension/solution was placed in the diffusion setup where gelatin paper was used as an equilibrium membrane and dialyzed against a 1.2 pH buffer for 2 h. At fixed time intervals, a 2 ml sample was withdrawn, and absorbance was measured at 305 nm. After 2 h, dialyzed against 7.4 pH buffer and 2 ml sample was withdrawn, and absorbance was read at 305 nm UV spectrophotometrically [19].

In vivo antihyperlipidemic drug action in albino rats

The polymeric NPs SE2 and IG2, which provided maximum *in vitro* drug release, were selected for the in vivo activity. The plasma lipid profiles were determined in the healthy albino rats weighing between 200-300 gm of either sex. For the study, animals were provided with regular pelletized food and water. The rats were divided into the following five groups: SE2, IG2, normal, control, and standard.

The course of treatment lasted 14 days. The normal group was fed with standard food pellets, the control group was fed with pure 2 ml coconut oil daily, and the standard group was fed pure FLS (2 mg/ kg body weight) daily. The test groups SE2 and IG2 were given the polymeric NP solution (2 mg/kg body weight) prepared in distilled water and distilled water with tween 80, respectively. Dosing was done daily for up to 14 days. The blood sample was collected on the 0, 3, 8 and 14 days by retro-orbital injection and centrifuged at 3000 rpm for 20 min at -20°C. The plasma was estimated for cholesterol and triglycerides using diagnostic kits (Erba Ltd. India). Fixed volumes mentioned in the leaflets of kits of the standard were prepared for reference, and a working reagent was added to the plasma of each group and kept aside for 10 min and simultaneously blank was prepared by omitting the sample and absorbed spectrophotometrically at 505 nm and 546 nm for cholesterol and triglycerides respectively [20].

Comparative pharmacokinetic drug release study

A comparative drug release kinetic study is carried out using Higuchi's and Kores Meyer and Poppa's models.

Higuchi Model-drug release from polymer matrix can be predicted

$$Q = K\sqrt{t}$$

Q = concentration of drug release;
K = slope;
√t = Square root of time

Graph plotted for Q v/s √t gives a straight line with slope K [18]. The data of in vitro release in an excel sheet model is checked using the concept mentioned above, or KinetDS3 software was utilized.

Kores Meyer Equation or Peppa's Model

$$\mathbf{C_t / C_\alpha = Kt^n}$$

Where,

Ct / Cα = The fraction of drug released at time 't'.
K = Constant indicating the properties of the drug/polymer system.
n = Diffusion exponent related to the mechanism of the release.

Above equation can be easily simplified,

$$\mathbf{\log C_t / C_\alpha = \log K + n \log t}$$

Using above equation and data of in vitro release in excel sheet model is verified Or KinetDS3 software was used [21].

Stability studies

Accelerated stability studies were performed to estimate the drug loss from the polymeric NPs. The study was carried out for the formulations of both methods, characterized by in vitro drug release. Formulations were stored at -2°C in the refrigerator and room temperature (29°C). They were then characterized for entrapment efficiency and *in vitro* drug release study after storing at room temperature and -2°C. They were estimated for one-month intervals for 3 months to verify both parameters [20].

Results

Solubility study of NPs

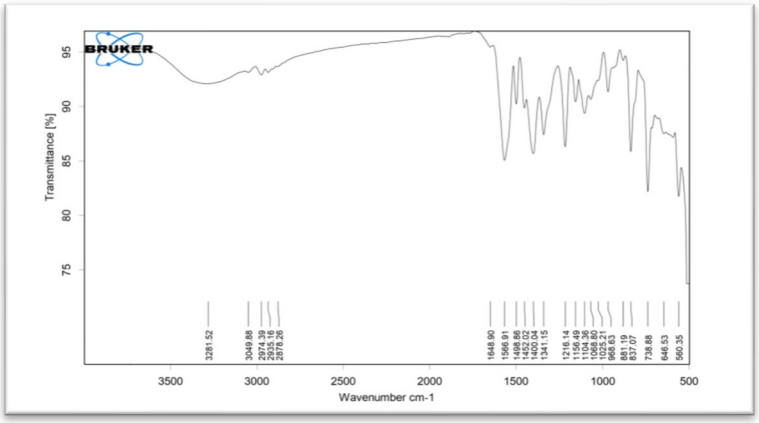
Table 3. Solubility studies of polymeric NPs.

Solvent name	NPs from SE method	NPs from IG method
Distilled water	Soluble	Insoluble
Methanol	Soluble	Soluble
Ethanol	Insoluble	Insoluble
Dimethyl formamide	Sparingly soluble	Sparing soluble

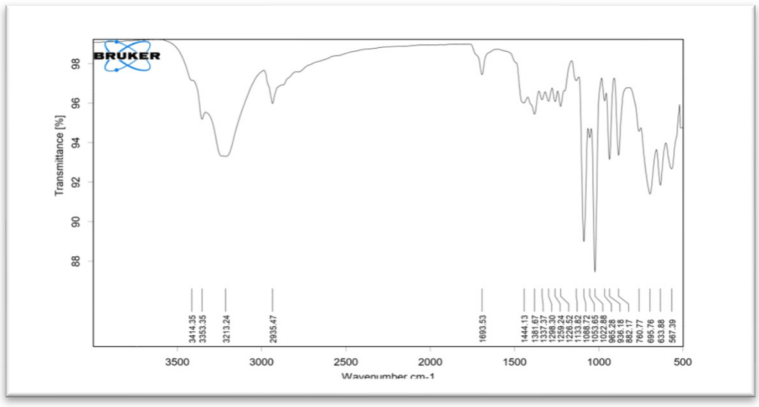
The solubility study for the polymeric NPs was based on the solubility profile of the Fluvastatin sodium drug. According to various literature, FLS is soluble in ethanol, methanol, and sparingly soluble in dimethyl formamide (Table 3).

FTIR studies

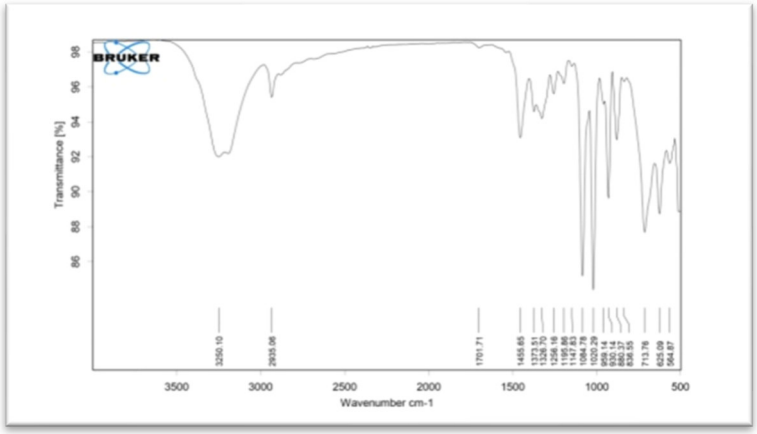
The FTIR estimation was performed to verify the compatibility between FLS and polymers used in the preparation. The spectrum obtained for a pure sample of FLS, SE3 and IG2 NPs, respectively, is shown in Figure 1.



FTIR Spectrum of Fluvastatin Sodium.



FTIR Spectrum of SE3 polymeric NPs



FTIR Spectrum of IG2 polymeric NPs

Figure 1. FT – IR spectrums of Pure drug, polymeric NPs from Solvent evaporation and Inotropic gelation method.

% Drug entrapment efficiency, Particle size, Zeta potential, and PDI

% Drug entrapment efficiency of SE polymeric NPs was obtained between the range of 46.8% to 86.1 %. In comparison, the DEE of IG NPs was found to be in the range of 44.6% to 70.2%, Graphically shown in Figure 2.

Particle size and PDI of the SE technique were found to be in the range of 271 to 313.3 nm and 0.453 to 0.781, respectively, whereas IG techniques fetched particles size and PDI in the range of 123.3 to 382 nm and 0.322 to 0.63 respectively.

Table 4. Measurement values of average particle size, PDI, Zeta potential, and %DEE.

Formulation Code		Particle size in nm	PDI	Zeta potential in mV	%DEE
SE	SE1	297.7	0.453	0.104	46.8
	SE2	271	0.562	26.1	82.3
	SE3	313.3	0.781	3.21	86.1
IG	IG1	123.3	0.422	11.1	44.6
	IG2	168.6	0.326	0.764	58.2
	IG3	382	0.630	9.09	70.2

Zeta potential can be used to represent a particle's surface charge. The results of the SE method were found to be in the range of +0.140 to + 26.1 mV, whereas the IG method results fall within the range of 0.764 to 11.1mV. The value for DEE, particle size, PDI, and Zeta potential is shown in Table 4.

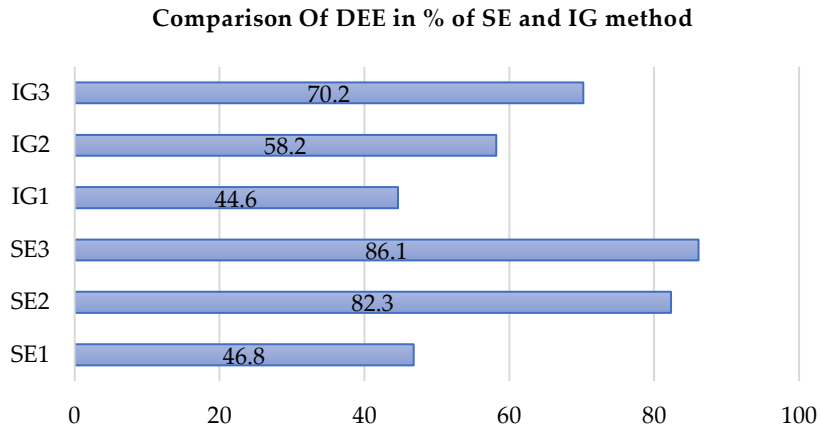
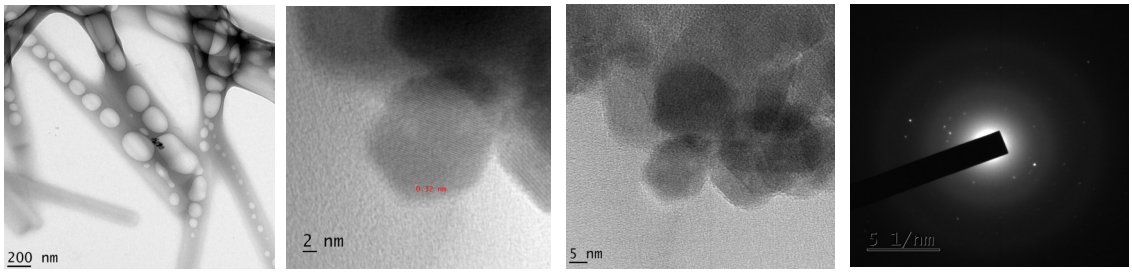


Figure 2. Comparison of % DEE of SE and IG methods.

Transmission electron microscopy (TEM)

High resolution transmission electron microscopy (HR-TEM) with the selected area electron diffraction (SAED) technique was used to characterize the SE3 polymeric NPs. The polymeric NPs had an elliptical shape with a smooth surface.

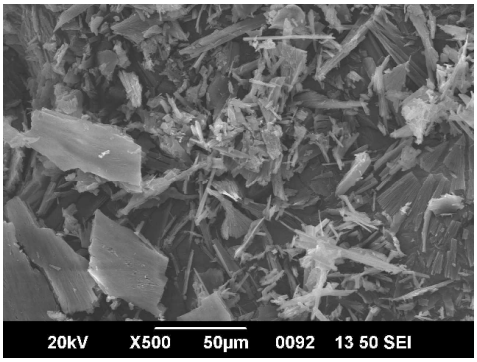


Elliptical shape Unidirectional planes Meso porosity SAED image

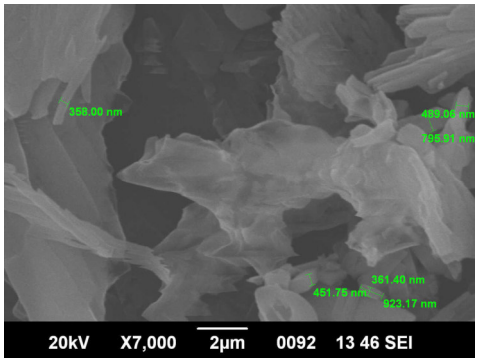
Figure 3. TEM with SEAD images of SE3 polymeric NPs.

Scanning electronic microscopy (SEM)

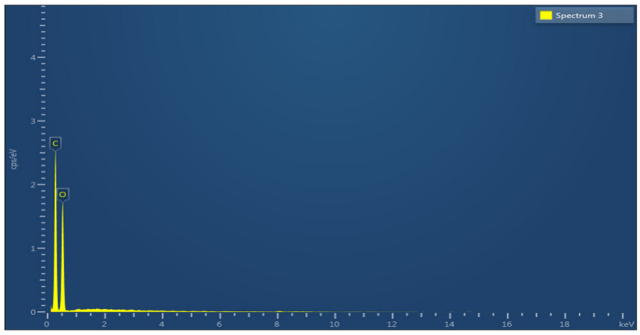
IG3 NPs were characterized by Field emission scanning electron microscopy (FE-SEM) with energy dispersive x ray analysis. The polymeric NPs from the IG method were found to be irregular in shape. The texture was found to be smooth.



Irregular shape



Smooth Texture



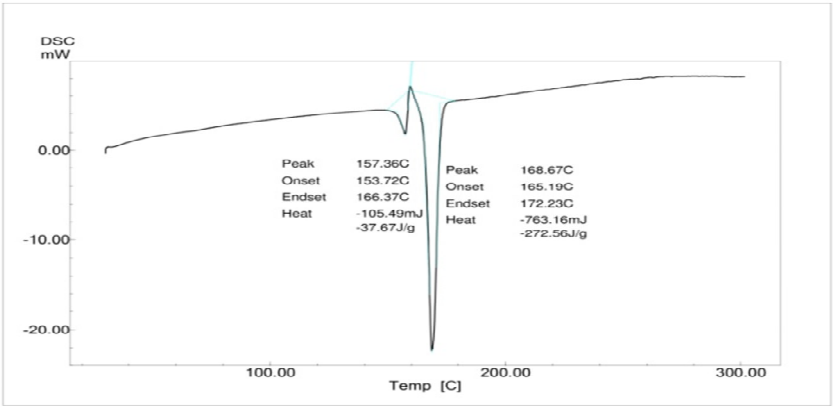
EDX image

Element	Line Type	Atomic %	Wt%
C	K series	57.02	49.9
O	K series	42.98	50.1
Total:		100	100

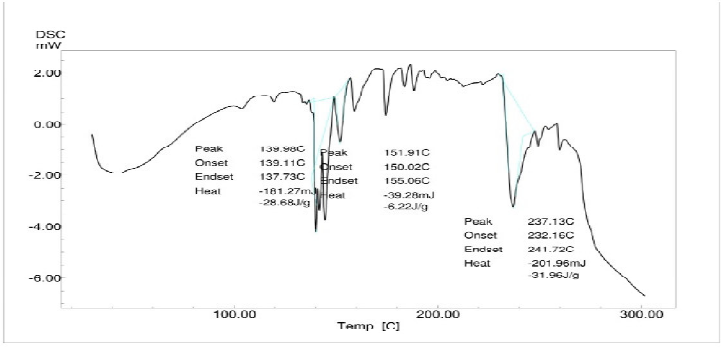
Figure 4. SEM with EDX image of IG3 polymeric NPs.

Differential scanning colorimetry (DSC)

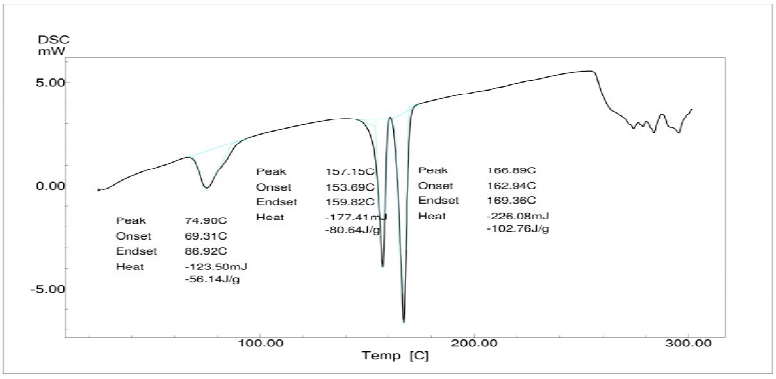
The DSC estimation was carried out for the samples compared with FLS. The nature of FLS found to be amorphous, and polymeric NPs also contained the amorphous nature. The results are shown in Figure 5.



DSC thermogram of Fluvastatin sodium



DSC thermogram of SE2 polymeric NPs

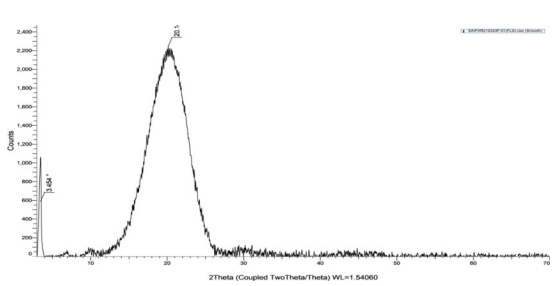


DSC thermogram of IG2 polymeric NPs

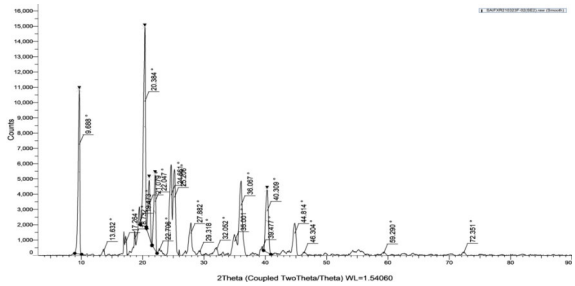
Figure 5. DSC thermograms of pure drug, polymeric NPs from solvent evaporation method and inotropic gelation method.

X-ray diffraction studies (XRD)

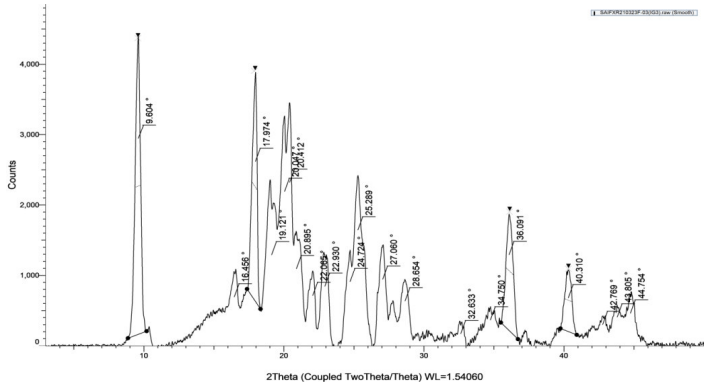
XRD was carried out for these two formulations against the pure drug. Results show that FLS and the prepared formulations are amorphous in nature.



(a) XRD of Fluvastatin sodium



(b) XRD of SE2 NPs



(C) XRD of IG3 NPs

Figure 6. XRD graphs of Fluvastatin sodium and NPs of SE and IG methods.

In vitro drug release studies

In vitro drug release studies were carried out by dialysis method using 1.2 pH buffer at first 2 h and in 7.4 pH buffer up to end. The percentage cumulative drug release of SE1, SE2, and SE3 was found to be 87.13%, 99.02%, and 55.75%, respectively. Meanwhile, IG1, IG2, and IG3 have shown drug release 79.05%, 84.6%, and 73.25%, respectively.

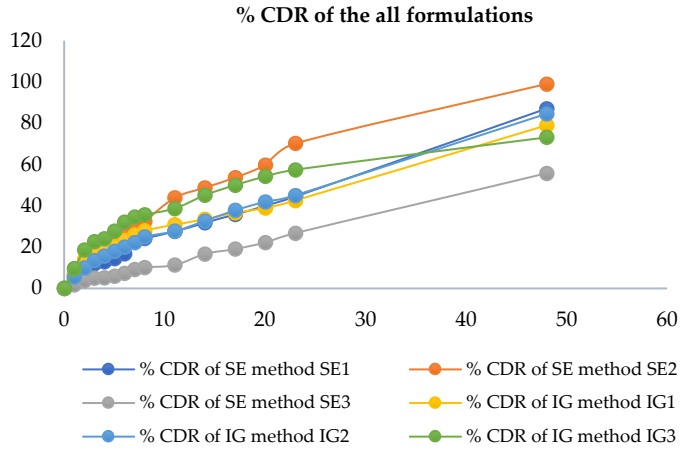


Figure 7. Comparison of in vitro study of SE and IG method.

Kinetic modelling on drug release study

The release data were subjected to Koresmeyer and Higuchi model using KinetdS3 software. The regression coefficient values obtained have shown in Table 5.

Table 5. Kinetic modelling on drug release study of polymeric NPs.

Formulation code		Zero Order (R^2 value)	Higuchi (R^2 value)	Peppas (R^2 value)	Best Fit Model
SE method	SE1	0.9892	0.771	0.9877	Both zero-order and peppas
	SE2	0.9310	0.8389	0.9902	Peppas
	SE3	0.9968	0.5563	0.9852	Zero-order
IG method	IG1	0.9744	0.9366	0.9743	Both zero-order and peppas
	IG2	0.9895	0.8522	0.9939	Peppas
	IG3	0.8614	0.9636	0.9720	Peppas

In vivo release study

The groups were estimated for total serum Triglyceride levels (TG) and Cholesterol levels (CH) over 14 days. In vivo drug release study showed that on the 0 days, the total serum TG and Ch of the normal group was more than that of the 3rd, 8th, and 14th days. Meanwhile, control and treated groups were too had more TG and Ch groups than on the 14th day. It was due to the random sampling of animals. On the 3rd day, the SE2 group had a slightly increased TG, and IG2 had slightly increased TG levels than standard due to the induction of hyperlipidemia along with the treatment. On the 8th day, the SE2 group decreased the TG level, whereas IG2 showed decreased Ch and TG levels than the standard. On the 14th day, both tested groups showed a significantly lower TG level than the standard. The stability studies showed that the formulation was stable in selected temperature conditions. Results are shown in Figure 8 and 9.

Stability studies

Accelerated stability studies were carried out by placing suspension/ solution of NPs at room temperature and -2 ℃ for 3 months. The % DEE of SE was found to be in the range of 39.2 to 83% at room temperature and 39.50 to 82.33% at -2℃; meanwhile, the IG method resulted in the range of 40.3 to 67% and 39.1 to 67.8%, respectively. The in vitro drug release of the SE method was obtained in the range of 49.99% to 96.23% at room temperature and 56.231 to 95.42% at -2℃, whereas the IG method had the range of 70.2 to 82.1% and 73.81 to 76.23%, respectively.

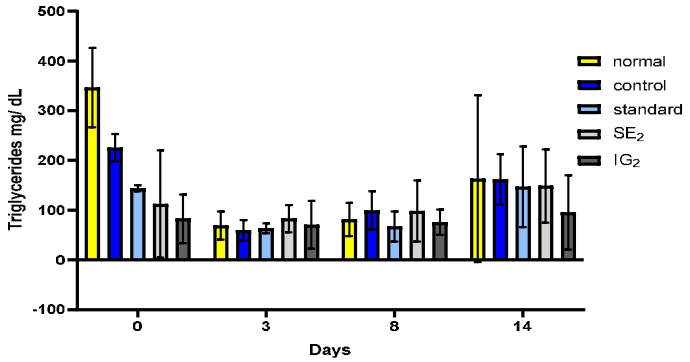


Figure 8. Serum triglyceride of rats group at different time intervals.

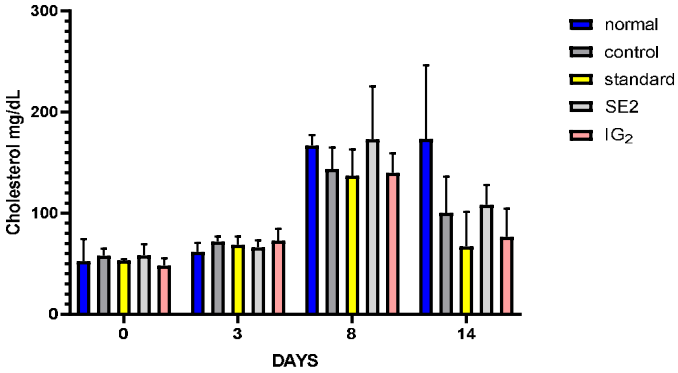


Figure 9. Serum cholesterol of rats group at different time intervals.

Discussion

The primary purpose of this work was to formulate Fluvastatin sodium-loaded polymeric NPs by solvent evaporation and inotropic gelation methods. These two methods were selected to compare the effects of polymeric NPs prepared from polymerization using monomers like inotropes and preformed polymers.

The FTIR peaks of the test samples were compared with a peak of FLS. The peak values present in the FLS graph approximately appeared in SE3. Furthermore, the IG2 showed H bonded N – stretching at 3250 cm^{-1}; C-N Stretching of aromatic amine at 1326 and 1256 cm^{-1} indicates that free amino groups remained while cross-linking as a result of rapid polymerization between chitosan and STPP shown in Figure 1 [22].

The comparison of the drug entrapment capacity of SE polymeric NPs range of 46.8% to 86.1% tells that the drug entrapment was increased by an increase in Drug and PVA and decreasing the Gum rosin concentration. But in the case of the inotropic gelation method, the drug entrapment efficiency found in the range of 44.6% to 70.2% indicates that an increase in the chitosan concentration increases the entrapment of the drug. From the %DEE, it can be predicted that the polymeric concentration increased, and better entrapment was found. Among SE and IG methods SE method exhibited higher entrapment efficiency shown in Figure 2. The value for DEE, particle size, PDI, and Zeta potential is shown in Table 4. The %DEE, particle size, PDI, and Zeta potential values are in the ideal range and hence fitted as the best model for preparing polymeric NPs for FLS [10].

TEM reveals that the polymeric NPs from the SE method were spherical and have mesoporosity, as light can slightly be passed in the section of the particles it was found to be mesoporous (Figure 3). The particles were found like grains and had unidirectional planes and patterns, Confirming that they are in amorphous form (Figure 3). SAED section of a sample was estimated for the diffraction where the d spacing value 0.2, which gives 20 planes which were confirmed to have fewer intensity peaks; hence the nature of SE2 was found to be amorphous (Figure 3) [23].

SEM reveals that the size of the IG2 was found to be irregular and smooth, and the particles are nonporous (Figure 4). The EDX value has shown in Figure 4. The atomic % and %wt. of C were found to be 57.02% and 49.9%, respectively; meanwhile, for O values were 42.98% and 50.1%, respectively. the cross linking between the chitosan and sodium tripoly phosphate in the inotropic gelation method was found to be rapid [24].

The DSC thermograph of SE2 showed endothermic peaks at 139.98℃, 151.91℃ and 237.13℃ (Figure 5) due to the melting of polymers. However, the peak of the drug corresponding to its melting point of 168℃ was absent, indicating that the NPs nature was amorphous and contained the uniformity of the drug in the polymer. DSC thermograph of IG2 involved a melting point of 74.90℃ nearer to the glass transition temperature, showing that the cross-linking has been taken between polymers and the presence of a peak at 166℃ corresponding to the melting point of the drug outlined that, NPs show amorphous nature but polymer linked to drug caused depression in the melting point and not uniformly dispersed in the linked matrix. However, no interaction was seen [12].

XRD of FLS showed peaks between 2θ of 10° to 30° (Figure 6a). SE2 sample showed peaks between 2θ of 10 to 70° (Figure 6b), and IG3 outlined peaks 2θ of 10° to 40° (Figure 6c) with poor periodicity [12]. Polymeric NPs obtained from both are amorphous in nature.

In vitro, drug release results signified that increased polymer results in slow drug release while more drug loading resulted in maximum drug release at the end of 48hrs. Comparing SE and IG techniques, the SE method fetched better release than IG. Figure 7 showed that the cumulative release for optimized formula SE2, which was found to be 99.02%, was better than IG2 [12]. The best pharmacokinetic fitting model was Kores Meyer and Peppa's model. The R2 values of optimized formulae SE2 and IG2 were found to be 0.9902 and 0.9932, respectively (Table 5) [20]. The in vivo activity showed better action for both methods by reducing cholesterol and triglyceride level. Formulations were stable at the selected conditions.

Conclusion

DEE of NPs was more for the SE method compared to the IG method. The polydispersity index of the SE and IG methods was less than 1. Hence, aggregation within the particles is less. The FT – IR studies indicate that the drug and polymers are compatible. Cross-linking of chitosan and STPP was seen, which was required for inotropic gelation. From SEM and TEM, it was concluded that NPs of the SE method have mesoporous particles, and particles prepared from the IG method are non-porous. By characterizing with XRD and DSC, it was found that NPs are amorphous. In vitro release study concluded that prepared polymeric NPs are a sustained release delivery system. The release kinetics shows that the model follows non – fickian mechanism and thus follows the zero-order release. In vivo release concludes that PNP reduces TG levels compared to Cholesterol. Stability studies showed that there were not many variations in the parameters within 3 months. Future aspects of this work are to approach better bioavailability in humans with less dose frequency.

Ethics approval and consent to participate

The present work was approved by the Institutional Animal Ethical Committee with the reference no: IAEC/HSKCOP/March 2020/PG8.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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